Phylogeography of *Chelonus insularis* (Hymenoptera: Braconidae) and *Campoletis sonorensis* (Hymenoptera: Ichneumonidae), Two Primary Neotropical Parasitoids of the Fall Armyworm (Lepidoptera: Noctuidae)

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ABSTRACT In a previous study, we observed no spatial genetic structure in Mexican populations of the parasitoids *Chelonus insularis* Cresson (Hymenoptera: Braconidae) and *Campoletis sonorensis* Cameron (Hymenoptera: Ichneumonidae) by using microsatellite markers. In the current study, we investigated whether for these important parasitoids of the fall armyworm (Lepidoptera: Noctuidae) there is any genetic structure at a larger scale. Insects of both species were collected across the American continent and their phylogeography was investigated using both nuclear and mitochondrial markers. Our results suggest an ancient north–south migration of *C. insularis*, whereas no clear pattern could be determined for *C. sonorensis*. Nonetheless, the resulting topology indicated the existence of a cryptic taxon within this later species: a few Canadian specimens determined as *C. sonorensis* branch outside a clade composed of the Argentinean *Chelonus grioti* Blanchard, the Brazilian *Chelonus flavicincta* Ashmead, and the rest of the *C. sonorensis* individuals. The individuals revealing the cryptic taxon were collected from *Trichoplusia ni* (Hübner) (Lepidoptera: Noctuidae) on tomato (*Lycopersicon* spp.) and may represent a biotype that has adapted to the early season phenology of its host. Overall, the loosely defined spatial genetic structure previously shown at a local fine scale also was found at the larger scale, for both species. Dispersal of these insects may be partly driven by wind as suggested by genetic similarities between individuals coming from very distant locations.

KEY WORDS parasitoids, mitochondrial DNA, internal transcribed spacer, Bayesian inference, spatial genetic structure

Patterns of intraspecific spatial genetic structure (SGS) reflect both historical and contemporaneous levels of gene flow among populations. Although it can be difficult to evaluate which factor, or combination of factors, best explains the observed SGS pattern (Barton and Wilson 1996, Waser and Strobeck 1998), it is important to combine our understandings of both the biology of a studied organism and its phylogeographical history to interpret dispersal patterns correctly. This is particularly true when working with species of economical importance.

Genetic structure can result from past or current barriers to dispersal, density fluctuations, dispersal patterns and mating systems (Chesser 1991a,b). In this study, we looked at the phylogeography of *Chelonus insularis* Cresson (Hymenoptera: Braconidae) and *Campoletis sonorensis* Cameron (Hymenoptera: Ichneumonidae), two important parasitoids of, in particular, the fall armyworm, *Spodoptera frugiperda* J.E. Smith (Lepidoptera: Noctuidae). The fall armyworm is one of the major pests of several economically important crops throughout the American continent (Kranz et al. 1977). It is attacked by several parasitoids (Molina-Ochoa et al. 2003, Hoballah et al. 2004, Murua et al. 2006, Wyckhuys and O'Neil 2006), and its management through biological control could be enhanced with knowledge on the population dynamics of these parasitoids and on their past and present genetic history.

The two parasitoids show different ecologies and behaviors. *Chelonus insularis* is an egg–larval parasitoid, whereas *C. sonorensis* attacks larvae. They can be expected to have adapted their searching behavior to the specific stage they attack. Indeed, *C. insularis* encounters patches of physically defenseless eggs, so it can lay dozens of eggs at once without having to move much. In contrast, *C. sonorensis* can lay rarely more than one egg per plant visited, because *S. frugiperda* larvae are often solitary due to their highly cannibal-

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Table 1. Origins of *C. insularis* specimens used for gene sequencing

Individual	Geographic coordinates	GenBank accession nos.		
		16S	*COI*	*Cyt b*
Argentina 1	27° 18′ S, 58° 55′ W	GQ252994	GQ252921	GQ252943
Argentina 2	27° 18′ S, 58° 55′ W	GQ252995	GQ252922	GQ252944
Argentina 3	27° 18′ S, 58° 55′ W	GQ252996	GQ252923	
Argentina 4	27° 18′ S, 58° 55′ W	GQ252997	GQ252924	GQ252945
Argentina 5	27° 18′ S, 58° 55′ W	GQ252998	GQ252925	GQ252946
Argentina 6	27° 18′ S, 58° 55′ W	GQ252999	GQ252926	GQ252947
Argentina 7	27° 18′ S, 58° 55′ W	GQ253000	GQ252927	GQ252948
Brazil 1	19° 28′ S, 44° 14′ W	GQ253001	GQ252928	GQ252949
Brazil 2	19° 28′ S, 44° 14′ W	GQ253002	GQ252929	GQ252950
Mexico, Colima 1	19° 16′ N, 103° 46′ W		GQ252930	GQ252951
Mexico, Colima 2	19° 16′ N, 103° 46′ W	GQ253003	GQ252931	GQ252952
Mexico, Jalisco 1	19° 52′ N, 103° 33′ W	GQ253004	GQ252932	GQ252953
Mexico, Jalisco 2	19° 52′ N, 103° 33′ W	GQ253005	GQ252933	GQ252954
Mexico, Chiapas 1	14° 43′ N, 92° 18′ W	GQ253006	GQ252934	GQ252955
Mexico, Chiapas 2	14° 43′ N, 92° 18′ W	GQ253007	GQ252935	GQ252956
Mexico, Chiapas 3	14° 43′ N, 92° 19′ W	GQ253008	GQ252936	GQ252957
Mexico, Chiapas 4	14° 43′ N, 92° 19′ W	GQ253009	GQ252937	GQ252958
Mexico, Veracruz 1	20° 29′ N, 97° 32′ W	GQ253010	GQ252938	GQ252959
Mexico, Veracruz 2	20° 29′ N, 97° 32′ W	GQ253011	GQ252939	GQ252960
Mexico, Puebla 1	20° 27′ N, 97° 38′ W	GQ253012	GQ252940	GQ252961
Mexico, Puebla 2	20° 27′ N, 97° 38′W	GQ253013	GQ252941	GQ252962
Mexico, Puebla 3	20° 27′ N, 97° 38′ W		GQ252942	GQ252963

istic behavior (Chapman et al. 2000). It is therefore expected that *C. sonorensis* has to travel much more than *C. insularis* to find a comparable number of hosts to parasitize. These different life histories may reflect in the phylogeographic patterns of the two species, and we might expect more structuring at a large scale in *C. insularis* than in *C. sonorensis. C. insularis* might tend to disperse less than *C. sonorensis;* therefore, more local structure may arise in this species.

The current study was also motivated by a proximate conclusion from a previous study (Jourdie et al. 2010) in which we documented a low level of genetic structure in these two species when performing small-scale analyses, by using microsatellite loci described in Jourdie et al. 2008), for populations of parasitoids collected in Mexico. To go one step further and to resolve patterns of dispersal throughout the New World (at the continental scale), we investigate in this paper whether this lack of structure at a small-scale also occurs at a larger scale (including other samples from the Neotropical as well as from Nearctic areas) using both nuclear and mitochondrial DNA sequences.

Materials and Methods

Sampling. Insects were collected across the American continent (Tables 1 and 2). *C. insularis* specimens from Mexico (states of Colima, Jalisco, Puebla, and Veracruz), Brazil (Sete Lagoas) and Argentina (Chaco province) were included in the study. Individuals of *C. sonorensis* came from Canada and Mexico.

Table 2. Origins of *Campoletis* spp. specimens used for gene sequencing

	Individual	Geographic coordinates	GenBank accession nos.			
			16S	*COI*	*28S*	*ITS*
C. sonorensis	Canada 1	42° 18′ N, 83° 2′ W	GQ252981	GQ252908	GQ252890	GQ252968
	Canada 2	42° 18′ N, 83° 2′ W	GQ252982	GQ252909	GQ252891	GQ252969
	Canada 3	42° 18′ N, 83° 2′ W	GQ252983	GQ252910	GQ252892	GQ252970
	Canada 4	42° 18′ N, 83° 2′ W	GQ252984	GQ252911	GQ252893	GQ252971
*	Canada 5	42° 18′ N, 83° 2′ W	GQ252985	GQ252912	GQ252894	
*	Canada 6	42° 18′ N, 83° 2′ W	GQ252986	GQ252913		
*	Canada 7	42° 18′ N, 83° 2′ W	GQ252987	GQ252914	GQ252895	
	Mexico, Jalisco 1	19° 52′ N, 103° 33′ W	GQ252988	GQ252915	GQ252896	GQ252972
	Mexico, Jalisco 2	19° 52′ N, 103° 33′ W	GQ252989	GQ252916	GQ252897	GQ252973
	Mexico, Jalisco 3	20° 28′ N, 102° 12′ W	GQ252990	GQ252917	GQ252898	GQ252974
	Mexico, Jalisco 4	20° 28′ N, 102° 12′ W	GQ252991	GQ252918	GQ252899	GQ252975
	Mexico, Nayarit 1	21° 05′ N, 104° 26′ W	GQ252992	GQ252919	GQ252900	GQ252976
	Mexico, Nayarit 2	21° 05′ N, 104° 26′ W	GQ252993	GQ252920	GQ252901	GQ252977
C. flavicincta	Brazil 1	19° 28′ S, 44° 14′ W	GQ252980	GQ252906	GQ252888	GQ252966
	Brazil 2	19° 28′ S, 44° 14′ W		GQ252907	GQ252889	GQ252967
C. grioti	Argentina 1	26° 15′ S, 65° 16′ W	GQ252978	GQ252904	GQ252902	GQ252964
	Argentina 2	26° 15′ S, 65° 16′ W	GQ252979	GQ252905	GQ252903	GQ252965

* Asterisk indicates individuals collected from *T. ni* (as opposed to *S. frugiperda* for the other individuals).

Table 3. Primers used for gene sequencing of *C. insularis* and *Campoletis* spp.

Gene		Sequence	Reference
28S	F: 28S D2 f	5′-AGA GAG AGT TCA AGA GTA CGT G-3′	Belshaw and Quicke (1997)
	R: 28S D2 r	5′-TTG GTC CGT GTT TCA AGA CGG G-3′	Campbell et al. (1993)
ITS	F: ITS 4	5′-TCC TCC GCT TAT TGA TAT GC-3′	White et al. (1990)
	R: ITS 5	5′-GGA AGT AAA AGT CGT AAC AAG G-3′	
Cyt b	F: CytB f	5′-TCT TTT TGA GGA GCW ACW GTW ATT AC-3′	Belshaw and Quicke (1997)
	R: CytB r	5′-AAT TGA ACG TAA AAT WGT RTA AGC AA-3′	
16S rDNA	F: 16S f	5′-CAC CTG TTT ATC AAA AAC AT-3′	Dowton and Austin (1994)
	R: 16S r	5′-CTT ATT CAA CAT CGA GGT C-3′	Whitfield (1997)
COI	F: C1-J-1859	5′-GGA ACT GGA TGA ACA GTA TAT-3′	Simon et al. (1994)
	R: C1-N-2191	5′-CCA GGT AAA ATT AAA ATA TAA ACT TC-3′	

Because no sequences are published for *Campoletis* except for *C. sonorensis*, other taxa of the same genus were included as well to evaluate the monophyletic status of the species and their relative position. Specimens of closely related species (i.e., *C. grioti* from Argentina and *C. flavicincta* from Brazil) were thus included. All individuals were obtained from *S. frugiperda*, except for three Canadian individuals (individuals 5, 6, and 7), which emerged from *Trichoplusia ni* (Hübner) (Lepidoptera: Noctuidae).

Laboratory Protocols. Total genomic DNA was extracted from the wasps' abdomen using the DNeasy tissue kit (QIAGEN GmbH, Hilden, Germany) following the manufacturer's instructions. Total DNA was resuspended in 200 μl of elution buffer (two elutions of 100 μl each).

Two mitochondrial regions (*16S* and *COI*) were amplified in both genera, and a third mitochondrial region (*Cyt b*) could be amplified in *C. insularis.* For *Campoletis* spp., two additional nuclear markers (*28S rRNA* and *ITS2*) also were amplified. Primer information is summarized in Table 3.

28S. The forward primer (28S D2 f) from Belshaw and Quicke (1997) and the reverse primer (28S D2 r) from Campbell et al. (1993) were used to obtain an amplified fragment of 427–510 bp. The polymerase chain reaction (PCR) conditions were 30 cycles of 98°C denaturation (15 s), 49°C annealing (30 s), and 72°C elongation (40 s) with an initial denaturation of 3 min at 93°C and a final elongation at 72°C for 3 min.

Internal Transcribed Spacer (ITS). The forward primer ITS 4 and the reverse primer ITS 5 (White et al. 1990) amplified a 1102–1517-bp fragment under the following polymerase chain reaction (PCR) conditions: initial denaturation of 1 min 30 s at 95°C; 35 cycles of 35 s at 95°C, 1 min at 53°C and 2 min at 72°C; final extension of 8 min at 72°C.

Cytochrome b (*Cyt b*). The wobble primers designed by Belshaw and Quicke (1997) were used to amplify a 231–424-bp fragment. The PCR conditions were 35 cycles of 92°C denaturation (1 min), 53°C annealing (1 min), and 72°C extension (1 min), with an initial denaturation of 1 min 30 s at 94°C and a final extension of 3 min at 72°C.

16S. The forward primer from Whitfield (1997) and the reverse primer from Dowton and Austin (1994) yielded an amplified fragment of 409–459 bp. The following PCR conditions were used: initial denaturation of 1 min 30 s at 94°C, followed by 35 cycles of 94°C denaturation (1 min), 53°C annealing (1 min) and 72°C extension (1 min), and a final extension of 3 min at 72°C.

Cytochrome c Oxidase Subunit 1 (*COI*). The mitochondrial DNA (mtDNA) *COI* was partially amplified using C1-J-1859 (forward) and C1-N-2191 (reverse) primers adapted for the bee (Simon et al. 1994). The amplified fragment was between 364 and 392 bp. It was obtained through the following PCR conditions: 1 min 30 s at 94°C, 35 cycles of 94°C denaturation (1 min), 50°C annealing (1 min) and 70°C extension (1 min), and final extension of 5 min at 70°C.

Data Analysis. The sequences were manually corrected using ChromasPro 1.41 (Technelysium Pty. Ltd., Queensland, Australia) and further aligned using ClustalW 1.4 (Thompson et al. 1994) implemented in BioEdit (Hall 1999). Analyses were run with the default parameters in Clustal (i.e., gap opening = 15, gap extension = 6.66, delay divergent % = 30, DNA transition weight = 0.50, and DNA weight matrix = IUB). Alignment was straightforward for all genes as there were no indels. BLAST searches were conducted on all sequences to check for possible contamination.

Selection of Outgroups. Sequences from closely related taxa to both *C. insularis* and *Campoletis* spp. were compared to determine which species were more suitable as outgroups. GenBank accession numbers of the sequences used are provided in Table 4. Sequences were obtained in Fasta format from GenBank and converted into Phylip format with ForCon 1.0 (Raes and Van de Peer 1999). Further alignment with *C. insularis* and *Campoletis* spp. matrices was performed using ClustalW 1.4 (Thompson et al. 1994) implemented in BioEdit (Hall 1999). A phylogenetic tree was then reconstructed using RAxML (Stamatakis 2006, Stamatakis et al. 2008) to determine the closest related outgroup taxa.

Phylogenetic Reconstructions. Once the outgroup was picked, a supermatrix composed of three and four nucleotide partitions respectively for *C. insularis* and *C. sonorensis* was built using Concatenate (Alexis Criscuolo, http://www.supertriplets.univ-montp2.fr/PhyloTools.php/). In the supermatrix, taxa in which no sequences were gathered for a given partition were coded as missing values for the corresponding cells

Table 4. GenBank accession numbers for *Cyt b*, *16S rDNA*, and *COI* sequences used to determine the most suitable outgroup taxa in the phylogenetic analyses

	Cyt b	*16S rDNA*	*COI*
Chelonus cautus 1			EF555604
Chelonus cautus 2			EF555605
Chelonus cautus 3			EF555606
Chelonus cautus 4			EF555607
Chelonus cautus 5			EF555608
Chelonus cautus 6			EF555609
Chelonus cautus 7			EF555610
Chelonus cautus 8			EF555611
Chelonus sp. 1		EU107068	EU106961
Chelonus sp. 2		EU107069	EU106962
Chelonus sp. 3		EU107070	EU106963
Chelonus sp. 4		DQ538554	DQ538844
Chelonus sp. 5			AF102723
Chelonus sp. 6		U68150	
Chelonus sp. 7		AY004037	
Chelonus sp. 8		AY004038	
Chelonus sp. 9		AF003512	
Chelonus sp. 10		AF029115	
Chelonus sp. 11			AY165727
Chelonus sp. 12	Z83639		
Chelonus inanitus 1		DQ538562	DQ538853
Chelonus inanitus 2		AJ535933	
Sathon falcatus	Z83638	AF102764	

(Wiens and Reeder 1995). Heuristic searches were performed using the three following criteria: maximum parsimony (MP), maximum likelihood (ML), and Bayesian inference. All trees generated were edited with FigTree version 1.1.2 (Andrew Rambaut, http://tree.bio.ed.ac.uk/software/figtree/).

Maximum Parsimony. MP analyses were performed using parsimony ratchet (Nixon 1999) as implemented in PAUPrat (Sikes and Lewis 2001). Based on recommendations by (Nixon 1999), ten independent searches were performed with 200 iterations and 15% of the parsimony informative characters perturbed. The shortest equally most parsimonious trees were combined to produce a majority-rule consensus tree. To assess the support at each node, non parametric bootstrap analyses were performed using PAUP* version 4.0b10 (Swofford 2002) with 1,000 replicates, tree bisection and reconnection branch swapping, simple sequence addition, MULTREES and holding 10 trees per replicate.

Maximum Likelihood. The ML analysis with the supermatrix treated as a single partition was performed using the RAxML web-server (http://phylobench.vital-it.ch/raxml-bb/) (Stamatakis 2006, Stamatakis et al. 2008). The model used by this software is by default GTR+G. *Treefinder* (Jobb 2008) also was used to perform a ML analysis and to check for congruency between the two algorithms.

Bayesian Inference. A Bayesian inference analysis (Nylander 2004) was performed on the supermatrix (with each DNA region represented as a separate partition), by using MrBAYES (Huelsenbeck and Ronquist 2001), with substitution models as estimated by MrAIC 1.4.3 (Nylander 2004). Two simultaneous Monte Carlo Markov chains were run for 5×10^5 generations, saving one tree every 100 generations. Stationarity was determined by looking at the average standard deviation of split frequencies. Trees recovered before stationarity being reached were discarded and Bayesian posterior probabilities (BPPs), representing the percentage of times each node was recovered, were calculated from a consensus of the remaining trees. Effective sampling size (ESS) for Markov chain Monte Carlo (MCMC) analyses in both species were calculated with Tracer 1.3 (Rambaut and Drummond 2005).

Results

Samples. Tables 1 and 2 provide details on how many individuals per species and per location were included in the analyses along with the GenBank accession numbers for all sequences used. All individuals emerged from *S. frugiperda*, except for three Canadian *C. sonorensis* wasps (individuals 5, 6, and 7), which emerged from *T. ni*. These *T. ni* larvae were collected from tomato plants only ≈100 m from the maize plants with the *S. frugiperda* larvae that provided the other four Canadian *C. sonorensis* individuals. Because the larvae were collected at the same time, it cannot be excluded that some of the parasitoids were from the same mother.

Alignments. Sequences are available in GenBank under accession numbers GQ252888 to GQ253013 (Tables 1 and 2). In *C. insularis*, two individuals failed to amplify *16S*, one failed to amplify *Cyt b*, whereas all individuals amplified for *COI*. For *Campoletis* spp., one individual failed in providing satisfactory amplification for both 16S and 28S, three were unsuccessful for *ITS*, whereas all individuals correctly amplified *COI*.

Selection of Outgroups. From the phylogenies obtained with the sequences published online, *Chelonus inanitus* (Hymenoptera: Braconidae) and *Venturia canescens* (Hymenoptera: Ichneumonidae) were chosen as outgroups for *C. insularis* and *Campoletis*, respectively (tree not shown). No *Cyt b* sequence was available for *C. inanitus* nor was the *ITS* sequence for *V. canescens*.

Phylogenetic Analyses. For *C. insularis*, the alignment consisted of 38 variable characters (of 1,190 in total), among which 36 characters were parsimony-informative. Under the MP criterion, the heuristic search resulted into 188 equally most parsimonious trees of 83 steps (consistency index [CI] = 0.904, retention index [RI] = 0.897, rescaled consistency [RC] = 0.811, and homoplasy index [HI] = 0.096). Regarding probabilistic criteria (i.e., ML and Bayesian inference), the best-fit model was the general time reversible (GTR). The topologies produced during the two ML searches (i.e., RAxML and TreeFinder) were identical and fully congruent with the Bayesian inference partitioned analysis (using a F81 best-fit model of evolution for all partitions). In this latter analysis, average standard deviation of split frequencies reached 1% after 150,000 MCMC generations, thus the first 1,500 first trees were discarded (burn-in). ESS was >500 for all parameters. For *Campoletis* spp., the alignment comprised 98 variable characters (of

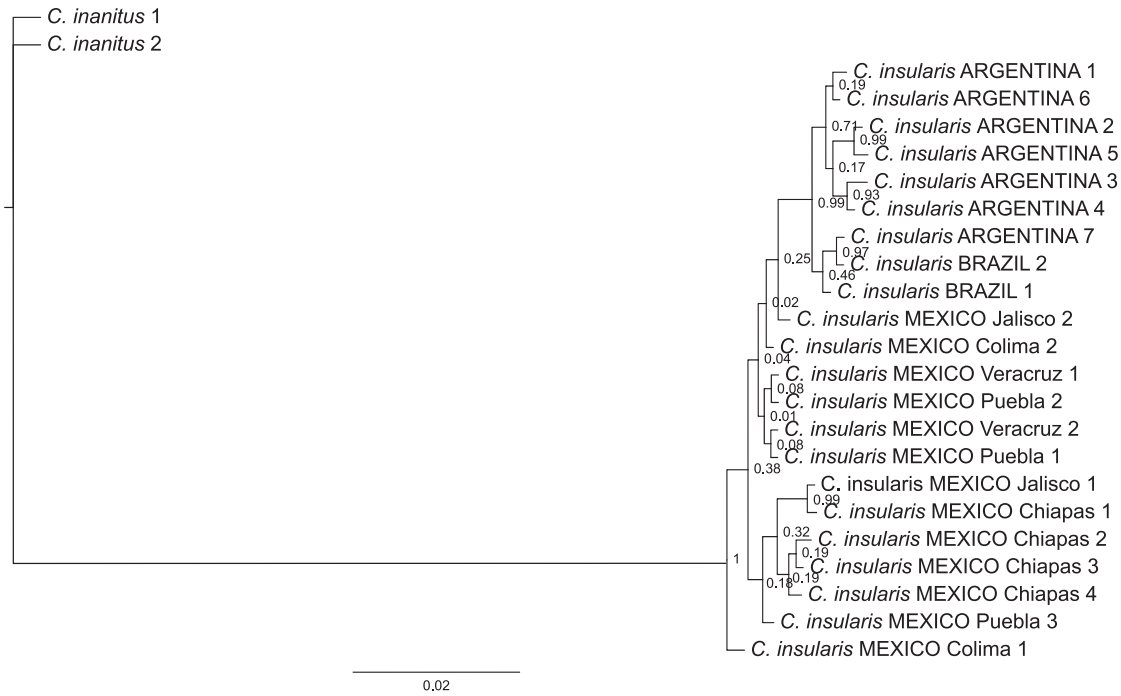

Fig. 1. Majority-rule phylogenetic tree resulting from the Bayesian inference analysis using a partitioned supermatrix approach (see Materials and Methods) for *C. insularis*. BPPs are indicated on each node. This tree was obtained from the analysis of three mitochondrial markers: *16S*, *COI*, and *Cyt b*.

2,591 in total), among which 43 characters were parsimony-informative. Under the MP criterion, the heuristic search resulted into 201 equally most parsimonious trees of 154 steps (CI = 0.948; RI = 0.929; RC = 0.880; HI = 0.051). Based on the probabilistic criteria, the best-fit model for *Campoletis* was the Hasegawa–Kishino–Yano (HKY) with an alpha parameter for the shape of the gamma distribution to account for among-site rate heterogeneity and a proportion of invariable site. The topologies produced during the two ML searches were identical and fully congruent with the Bayesian Inference partitioned-analysis, in which the best-fit model for individual DNA partitions were HKY+G for *16S*, HKY for *28S*, HKY+I for *COI*, and K2P for *ITS*. In this latter analysis, average standard deviation of split frequencies reached 1% after 100,000 generations, thus discarding the first 1,000 trees. ESS was >200 for all parameters.

Topologies. The topologies unraveled with the different methods were highly similar therefore we show only the phylogenetic trees resulting from the Bayesian inference analyses (see Fig. 1 for *C. insularis* and Fig. 2 for *Campoletis* spp.). For *C. insularis*, the group including individuals coming from Argentina and Brazil is monophyletic and nested in the Mexican group (BPP = 0.99). Within the South-American group, no clear structure can be observed. The Mexican group shows no clear structure either and exhibits paraphyletic origins together with the South-American group. However, this paraphyly is not well supported (BPP = 0.38). For *Campoletis* spp., our results demonstrate that *Campoletis sonorensis* forms a paraphyletic group with well supported clades (BPP = 1). Similarly, *C. grioti* exhibits paraphyly (BPP = 1). In contrast, *C. flavicincta* is monophyletic (BPP = 1). No clear geographic structure is observed in *C. sonorensis*. However, the three Canadian individuals collected on *T. ni* come out in an entirely separate clade (BPP = 0.98).

Discussion

In *C. insularis*, the group of individuals coming from South America is clearly nested into the Mexican group, implying that South American wasps might derive from Mesoamerican ancestors. The monophyly of the South American group contrasts with the paraphyletic origins of the Mexican group. South American populations may therefore have arisen from a single colonization event. This result should, however, be taken with caution because the choice of the outgroup as *C. inanitus* (i.e., the closest to *C. insularis* in our phylogeny) has been based only on sequences of about a dozen published *Chelonus* taxa, whereas this genus is estimated to comprise several hundreds of species. Nonetheless, it is worth noting that the Mexican group is as diversified as the South American group, which, in conjunction with the previous notion, argues for a possible north–south migration. Yet, a more extensive sampling in North America, as well as a more accurate identification of the closest outgroup (once a complete phylogeny of *Chelonus* has been

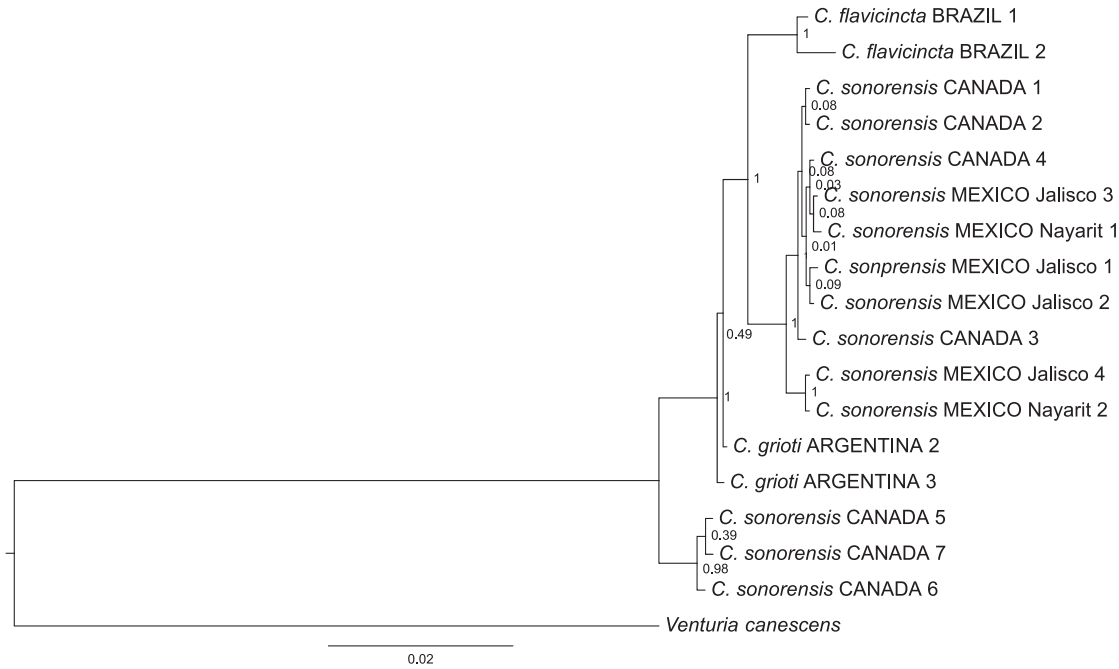

Fig. 2. Majority-rule phylogenetic tree resulting from the Bayesian inference analysis using a partitioned supermatrix approach (see Materials and Methods) for *Campoletis* spp. BPPs are indicated on each node. This tree was obtained from the analysis of two mitochondrial (*16S* and *COI*) and two nuclear (*28S rRNA* and *ITS2*) markers.

published) will be necessary to determine the exact geographic origins of this species. The high level of admixture at a regional scale (i.e., the very weak local fine structure) indicates considerable gene flow between populations. For instance, individual one from Jalisco and individual one from Chiapas come out together in a very well supported clade (BPP = 0.99) (i.e., sequences from the two individuals are identical in all partitions) despite being geographically distant from >1,000 km. Similarly, individual seven from Argentina and individual two from Brazil are identical for all partitions even though they come from two distant locations separated by >1,300 km.

Regarding *C. sonorensis*, the data strongly support the paraphyly of this taxon. The apparent paraphyly of *C. grioti* might be mostly due to a small sampling size. Indeed, it is not supported by Bayesian posterior probability (BPP < 0.5). Nonetheless, there was a striking difference between the three Canadian individuals (5, 6, and 7), which were collected on *T. ni*, and the other Canadian individuals that emerged from *S. frugiperda* (BPP = 1), implying that use of different host species may strongly affect evolutionary processes and may have lead to genetic differentiation. Offspring of wasps collected from the two hosts also were compared morphologically and all confirmed to be *C. sonorensis* (Dr. A. Bennett, Canadian National Collection). In the laboratory, the two lineages (i.e., *C. sonorenis* specimens on *S. frugiperda* and *C. sonorensis* specimens on *T. ni*) were found to successfully parasitize the two hosts, but they both did better on *S. frugiperda* (H.M., unpublished data). This would explain why *C. sonorensis* has been very rarely reported as parasitizing *T. ni* (Murillo 2008). However, in southwestern Ontario, Canada, from where we obtained our Canadian individuals, *C. sonorensis* is the most abundant parasitoid of *T. ni* on tomato in the field and greenhouses. Therefore, we speculate that the clear separation in Canada of *C. sonorensis* from *S. frugiperda* and *C. sonorensis* from *T. ni* is the result of an ancient adaptation of a biotype to the early seasonal occurrence of *T. ni*, perhaps through a synchronized diapause. *Trichoplusia ni* is present in high numbers from May, whereas *S. frugiperda* is present only starting midsummer (Murillo 2008).

Molecular tools once again proved useful to bring cryptic species to the fore, as it was the case in several previous studies (Hebert et al. 2004, Smith et al. 2006, Burns et al. 2008). There was, however, no indication of local fine structure. Indeed, *C. sonorensis* individuals coming from close geographic locations, i.e., individuals three and four from Jalisco (Mexico), and individuals one and two from Nayarit (Mexico), pair-up in different very well-supported clades (BPP = 1). Moreover, if we only take into consideration individuals of *C. sonorensis* emerging from *S. frugiperda*, there is no evidence for structure at the scale of the North American continent.

Hence, the low level of genetic structure observed at the local Mexican scale by using microsatellites (Jourdie et al. 2010) was confirmed at a larger scale with the use of other sequence markers. Interpopulation movements seem to be very important even over very large distances. Whereas patterns of popu-

lation structure found in different Mesoamerican insect pests have been explained by farmers' practices and contemporaneous trade of plant parts such as seeds (Alvarez et al. 2007, Restoux et al. 2010), human-mediated dispersal can be ruled out in the context of these two parasitoid species, which are not able to disperse in association with the plant. However, wind is a likely mode of dispersal for these insects as they are too small and fragile to fly over such distances. Wind is known to play an important role in dispersal of small flying insects (Compton 2002). Dispersal by moving air largely dictates direction and distance of migration to small insects, which can be expected to reflect in the genetic population structure (Dudley 2000).

The observed absence of genetic structure might also result from a keen ability of these insects to adapt to environmental changes and therefore to readily invade new environments (Hengeveld 1989, Kareiva 1996). This adaptability enhances their potential as effective biological control agents in new areas of release. Finally, cryptic taxa brought to evidence in this study may have different host ranges and represent new candidates for biological control.

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